CLAIMS

1. A method for identifying or otherwise detecting a nucleotide repeat region, characterized by a particular length, in a nucleic acid molecule, said method comprising annealing to a single stranded template from said nucleic acid molecule a set of oligonucleotides, said set comprising at least two flanking oligonucleotides which are capable of annealing to nucleotide sequences on the template nucleic acid molecule flanking the nucleotide repeat region and at least one spacer oligonucleotide capable of annealing to a nucleotide sequence defining all or part of the nucleotide repeat region and wherein one of said flanking oligonucleotides is labelled with a capturable moiety and the other of said flanking oligonucleotide is labelled with a detectable moiety and subjecting the annealed molecules to a ligation reaction sufficient to permit ligation of two or more oligonucleotides if ligatably adjacent to each other and then subjecting the ligation product to conditions to facilitate attachment of the capturable moiety to a binding partner immobilized to a solid support and then subjecting the immobilized molecule to denaturing conditions to separate the template nucleic acid molecule from the annealed, potentially ligated oligonucleotides and then screening for said detectable moiety on the solid support and wherein the presence of said detectable moiety is indicative that said spacer oligonucleotide is ligated to the flanking oligonucleotides, the length of said spacer oligonucleotide thereby corresponding to the length of the nucleotide repeat region.

2. A method of identifying or otherwise detecting a nucleotide repeat region characterized by a particular length in a nucleic acid molecule, said method comprising annealing to a single stranded template form said nucleic acid molecule at least two flanking oligonucleotides which flank the putative nucleotide repeat region to be identified and, in a multiplicity of separate reactions, a spacer oligonucleotide of a defined length in each separate reaction which spacer oligonucleotide anneals to all or part of the nucleotide sequence between said flanking oligonucleotides wherein one of said flanking oligonucleotides is labelled with a capturable moiety and the other of said flanking oligonucleotide is labelled with a detectable moiety and subjecting said annealed molecules to ligation reactions and attachment conditions such that the oligonucleotide

comprising a terminal capturable moiety anchors the annealed, potentially ligated nucleic acid molecule to a solid support; subjecting said anchored nucleic acid molecule to denaturing means such that the template nucleic strand of the nucleic acid molecule separates from the annealed oligonucleotides and then screening for said detectable moiety on a flanking oligonucleotide wherein the presence of a detectable signal is indicative that the three oligonucleotides are in tandem ligatable arrangement wherein the spacer oligonucleotide in the reaction giving the signal corresponds to the length of the nucleotide repeat region.

3. A method of identifying or otherwise detecting a nucleotide repeat region characterized by a particular length in a nucleic acid molecule, said method comprising annealing to a single stranded template form said nucleic acid molecule at least two flanking oligonucleotides which flank the putative nucleotide repeat region to be identified and, in a multiplicity of separate reactions, a spacer oligonucleotide of a defined length in each separate reaction which spacer oligonucleotide anneals to all or part of the nucleotide sequence between said flanking oligonucleotides wherein one of said flanking oligonucleotides is labelled with a capturable moiety and the other of said flanking oligonucleotide is labelled with a detectable moiety and subjecting said annealed molecules to ligation reactions and attachment conditions such that the oligonucleotide comprising a terminal capturable moiety anchors the annealed, potentially ligated nucleic acid molecule to a solid support; subjecting said anchored nucleic acid molecule to denaturing means such that the template nucleic strand of the nucleic acid molecule separates from the annealed oligonucleotides and then screening for said detectable moiety on a flanking oligonucleotide wherein the presence of a detectable signal is indicative that the three oligonucleotides are in tandem ligatable arrangement wherein the spacer oligonucleotide in the reaction giving the signal corresponds to the length of the nucleotide repeat region.

4. A method according to Claim 1 or 2 or 3 wherein the spacer oligonucleotide region is from about 2 to about 400 nucleotides in length.

5. A method according to Claim 4 wherein the spacer oligonucleotide region is from about 2 to about 200 nucleotides in length.

6. A method according to Claim 5 wherein the spacer nucleotide region is from about 2 to about 120 nucleotides in length.

7. A method according to Claim 1 or 2 or 3 wherein the template nucleic acid molecule is from a nucleic acid molecule which has been subject to amplification.

8. A method according to Claim 7 wherein the amplification reaction is PCR, rolling circle amplification or Qβ replicase-based amplificaiton.

9. A method according to Claim 8 wherein the amplification reaction is PCR.

10. A method according to Claim 1 or 2 or 3 wherein the nucleic acid molecule is DNA.

11. A method according to Claim 10 wherein the nucleic acid molecule is cDNA.

12. A method according to Claim 1 or 2 or 3 wherein the nucleotide repeat region is characteristic of a new degenerative disease.

13. A method according to Claim 12 wherein the neurodegenerative disease is fragile X syndrome, Huntington's disease or muscular dystrophy.

14. A method according to Claim 13 wherein the neurodegenerative disease is Huntington's disease.

15. A method according to Claim 1 or 2 or 3 wherein the solid support is glass or a polymer.

16. A method according to Claim 15 wherein the polymer is cellulose and its derivatives, ceramic material, nitrocellulose, polyacrylamide, nylon, polystyrene and it derivatives, polyvinyl chloride or polypropylene.

17. A method for determining the length of a nucleotide repeat region such as in the form of a microsatellite in a target nucleic acid molecule, said method comprising the steps of:-

(i) obtaining a sample of said target nucleic acid molecule;

(ii) optionally amplifying the repeat region on said target nucleic acid molecule;

(iii) subjecting the target nucleic acid molecule to denaturing conditions to yield a single stranded template carrying the repeat region;

(iv) annealing to said template three oligonucleotides separating, sequentially or simultaneously wherein two oligonucleotides are flanking oligonucleotides which are capable of annealing to the template at positions flanking the nucleotide repeat region and the third oligonucleotide is of a defined length and complementary to the nucleotide repeat region and wherein one of said flanking oligonucleotides is labelled at one end with a capturable moiety and the other flanking oligonucleotide is labelled at an end opposite to the first mentioned flanking oligonucleotide with a detectable moiety;

(v) subjecting the annealed oligonucleotides-template complex to ligation conditions such that the flanking oligonucleotides ligate to

the spacer oligonucleotide if the spacer oligonucleotide is ligatably adjacent the flanking oligonucleotides;

(vi) subjecting the ligation product to anchoring conditions to capture the flanking oligonucleotide carrying the capturable moiety to a solid support;

(vii) subjecting the captured ligation product to denaturing means to release the template; and

(viii) screening for an identifiable signal wherein the presence of a signal is indicative of a spacer oligonucleotide corresponding to the length of the nucleotide repeat region.

18. Use of ligase assisted spacer addition (LASA) in the identification of a nucleotide length polymorphism in an animal or human subject.

19. Use according to Claim 18 wherein the polymorphism is associated with a neurodegenerative disease.

20. Use according to Claim 19 wherein the neurodegenerative disease is fragile X syndrome, Huntington's disease or muscular dystrophy.

21. Use according to Claim 20 wherein the neurodegenerative disease is Huntington's disease.

22. A composite nucleotide sequence comprising the structure

$$[x_I x_{II} \ldots x_n]\ (\bullet)_a\ [y_I y_{II} \ldots y_m]\ (\bullet)_b\ [z_I z_{II} \ldots z_o]$$

wherein $[x_I x_{II} \ldots x_n]$ and $[z_I z_{II} \ldots z_o]$ are oligonucleotides of length n and o, respectively, capable of annealing to two nucleotide sequences flanking a nucleotide repeat region on a nucleic acid molecule;

$[y_I y_{II} \ldots y_m]$ is an oligonucleotide of length m and capable of annealing to a nucleotide repeat region between the two flanking nucleotides $[x_I x_{II} \ldots x_n]$ and $[z_I z_{II} \ldots z_o]$;

$(\bullet)_a$ and $(\bullet)_b$ represent phosphodiester bonds between adjacent nucleotides wherein $_a$ and $_b$ may be the same or different and each is 0 or 1 and wherein when $_a$ and/or $_b$ is 0, the adjacent oligonucleotides are not ligated together;

wherein said composite oligonucleotide is formed by the process comprising annealing x, y and z separately or simultaneously to a singled stranded template nucleic acid molecule comprising a nucleotide repeat region wherein x and z anneal to regions flanking y, subjecting the molecules to ligation to generate $(\bullet)_a$ and $(\bullet)_b$ wherein $_a$ and $_b$ are both 1 if y is ligatably adjacent x and z on the template; immobilizing the ligated product to a solid support and subjecting the immobilized product to denaturing conditions to remove the template and then detecting the presence of the composite oligonucleotide wherein the presence of a composite oligonucleotide is indicative that y is ligatable adjacent x and z.

23. A method for discriminating between nucleotide repeat regions characterized by particular lengths in a nucleic acid molecule, said method comprising annealing to a single-stranded template from said nucleic acid molecule a set of oligonucleotides wherein one oligonucleotide anneals upstream of a putative nucleotide repeat region and is of a length which is shorter than the repeat region or is longer than said repeat region and a second oligonucleotide anneals downstream of said repeat region and wherein one of said upstream or downstream oligonucleotides is labelled with a capturable moiety and the other of said upstream of downstream oligonucleotides is labelled with a detectable moiety and then subjecting said upstream oligonucleotide to nucleotide extension conditions whereby if the upstream oligonucleotide is shorter than the

repeat region, the extension product becomes ligatably adjacent the downstream oligonucleotide whereas if the upstream oligonucleotide is longer than the repeat region, then ligation is not possible with said downstream oligonucleotides such that upon ligation and immbolization to a solid support, the presence or absence of a detectable signal is indicative of an upstream oligonucleotide of a particular length and thereby a repeat region of a particular length.

24. A method according to Claim 23 wherein the method is conducted in duplicate with one or two upstream oligonucleotides wherein one of said oligonucleotides is potentially longer than said repeat region and the other oligonucleotide is potentially shorter than said repeat region and/or both oligonucleotides are potentially shorter than said repeat reigon.

25. A method according to Claim 23 or 24 for the detection of a neurodegenerative disease.

26. A method according to Claim 25 wherein the neurodegenerative disease is Huntington's disease.

27. A computer program-assisted method for detecting or identifying a nucleotide length polymorphism, said method comprising:-

(i) means to perform LASA or a related method; and

(ii) data processing means to record the presence of an identifiable signal and correlating same to the size of a spacer oligonucleotide.